The Spread of Potato Scab in Soil

by

Potato Plant Humus

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The accumulation of a disease-producing type of organism in a soil that is planted for a long period of years to one crop is one of the most dangerous factors in the spread of a plant disease. The simplest recommendation would be, of course, to practice a short or long rotation, but in many cases it could not be followed since the location of the land or the type of soil makes continuous cropping with one plant almost a necessity.

This very important practical aspect compels the introduction of other means of disease control than crop rotation. The complete life history and habits of the pathogene must be determined, in order to discover some phase of its life cycle in which it is vulnerable to attack. It may then be possible to break a link in the infection chain which carries the disease from one year to the next.

Parasites May Live in the Soil as Saprophytes

Parasitic fungi and bacteria may not only persist in the soil in a resting form, but may also live saprophytically on plant remains. The fusaria, for example, play a useful rôle in the soil during their saprophytic life by decomposing the pectin which holds the cells together. The enzyme, pectinase, by which this work is accomplished becomes a danger, however, when the fungus comes into contact with living cells.

The common (or corky) scab of potatoes and other plants is one of the commonest of the diseases which attack the underground parts of plants. The organisms which stimulate the corky layer of tubers to abnormal growth belong to the actinomycetes. Actinomycetes seem to be normal soil microflora, of which they constitute from five to thirty percent.

While the actinomyces group is usually classed with the bacteria, its members more nearly resemble some of the molds in morphology and physiology. Reproduction is carried out by fragmentation of the living protoplasm (Lieske, 1921), the protoplasm being enclosed in a sheath. The fragments may be so short that they are almost spherical or they may be of considerable length. Inside the hyphae,

* The author acknowledges with appreciation the help of two of his former students: Mr. Robert Bickford, who assisted in the laboratory and cared for the field experiment, and Mr. Norman Stoddard, who prepared the potato humus beds.

which extend aerially, the fragments may be regular (conidia) and may coil in fairly regular loops. But any part of a filament may serve as a reproductive body.

Fragmentation occurs in old cultures and is the result of unfavorable growth conditions, especially a lack of water. Conidia or resting bodies, which contain less water than the vegetative mycelium, are able to withstand unfavorable conditions. But to germinate, they must first absorb water through a very impermeable membrane and so are slower to start growth than pieces of vegetative mycelium which contain a more nearly normal water content.

Lutman, Livingston, and Schmidt (1936) found bits of mycelium in soil smears and suggested that the actinomycetes must be present in some more active form than that of spores or conidia.

Starkey (1938), using the Cholodny method, has reproduced convincing photographs of the soil actinomycetes in the rhizosphere, which show not only filaments but also chains of conidia, and germinations from conidia. In the Cholodny method for the examination of soil flora, a sharp vertical cut is made into the soil to be examined and a glass slide is pressed against the fresh surface. The slide is allowed to remain in this position for some time (from 36 to 53 days), after which it is removed and the organisms on its surface are stained. The claim is made that by this method a truer picture of the species and habits of a soil microflora is reproduced on the surface of the slide than is obtained by the smear technic, in which the organisms are broken apart and are not in their normal relationships to the soil particles or to each other.

Dippenaar (1933), reversing the Cholodny technic, has dried actinomyces spores on slides and then inserted them into an autoclaved loam compost which contained water up to 45, 65, or 85 percent of its water-holding capacity. In the two drier soils, the spores germinated and grew into a vegetative mycelium, the loam compost containing enough nutrient substances to produce a tangled growth of hyphae. The conclusion to be drawn is that in rich soils containing water equal to about one-half to two-thirds of their water-holding capacity, the scab organisms continue to grow vegetatively.

Tropical soils normally contain no humus although exceptions occasionally occur. The condition of actinomycetes in these soils is described by Subrahmanyan (1929) as follows:

"In the normal soil actinomyces are found almost exclusively in the form of conidia, and that, when they occur vegetatively as they do in some rare cases, their mycelia are largely present on decomposed plant residues and similar forms of organic matter which are generally removed during sampling, and not on the soil itself. Since

the conidia resemble many of the commoner forms of bacteria in shape and size, no direct method of counting them, stained or otherwise, will be possible. The plate method alone can provide some information regarding the numbers and distribution of *actinomyces* in soil."

Subrahmanyan in the same paper points out the difference in the time required for germination by hyphae and conidia of *Actinomyces chromogenus* and *Act. reticuli* on agar in petri plates. The hyphal fragments germinated in 24 hours, but the conidia germinated only after six days.

The writer (1941b, 1945) presented a new viewpoint on the mode of life of the actinomycetes in potato tubers and plants and in the soil. He stated that the organisms of potato scab live in the intercellular matrix, which is filled with various pectin compounds, where they may be traced in stained potato tuber sections. The writer made the suggestion, therefore, that these organisms may persist saprophytically in soil on the intercellular pectin of plant fragments.

Scab Organisms May Infect Other Parts of the Potato Plant than the Tubers

Scab organisms are considered by many investigators to be parasitic inhabitants only of the tuber cork and cork cambium. These organisms spread into the soil from conidia which appear as a gray mold on scab lesions. It is believed, therefore, that the removal of all scabby tubers from a field will eliminate all actinomyces mycelium present in the tubers, and leave only conidia in the soil to infect the succeeding crop. In this theory the writer does not concur. Potatoes become scabbier if grown continuously on the same soil (Lutman, 1941a).

Lesions on underground potato stems were investigated by Edson and Shapovalov (1918), but no infections of tubers by the ordinary scab organisms were noted.

The importance of infection of the lenticels on the potato stem and on the stolons does not seem to have impressed scab investigators. In studies on the influence of soil temperature on potato scab, however, Jones, McKinney, and Fellows (1922) did note: "In the experimental work involving the use of the scab organism, it has been found that the underground bases of the stems of the potato plant and also the stolons develop severe scab lesions which usually originate in the lenticels. This condition seldom occurs at soil temperatures below 24° C. and it becomes more pronounced as the temperature rises. As in the case with tubers, stems are clean and white in the control pots not containing the scab organism."

Beijerinck (1900) described actinomycetes in the roots of various plants, mostly trees, but unfortunately left no drawings. In another paper, he stated (1913): "That this *Actinomyces* must belong to another species than *Actinomyces chromogenus*, so common in our environment, is obvious. The latter, namely, is characterized by the production of a dark brown pigment from pepton (but not from tyrosin) in which, as I have formerly shown, under circumstances chinon may be found."

Scab lesions similar to those of the potato are known to occur on the roots of a variety of cultivated plants, including the garden beet, radish, peanut, carrot, parsnip, and eggplant, and on weeds such as *Amaranthus retroflexus*, but the relation of these organisms to those which occur on potatoes is not clear and the experiments on actinomyces strains from various sources conducted by Kenknight (1941) were very inconclusive.

The potato scab organism occurs in virgin soils in considerable numbers. In fact, Pratt (1918) has shown a higher percentage of scab on potatoes planted on virgin desert land of Idaho than on potatoes grown on the same land after other crops had been grown there, suggesting that these crops had introduced some factor which checked the numbers or virulence of the scab organisms.

Strands of fungi have been observed in root and underground stem tissues of a number of plants. The extensive work that has been done in France on tuberization and fungi is too lengthy to discuss but may be found in the paper by Costantin and Magrou (1935), in Magrou's papers on tuberization and the potato (1921, 1940), and in Rayner's monograph (1927) on mycorrhiza in general.

In the work which follows, the writer presents the possibility that parts of the potato plant other than the tubers may serve to carry the scab infection. No attempt has been made to differentiate between scab and non-scab organisms in the soil; both grow well on ordinary bacteriological media, the pathogenic species being so little different from the saprophytic ones that no laboratory differentiation can be found and reliance must be placed on inoculation. In fact, the separation of actinomyces species by the most elaborate bacteriological technic by competent observers such as Conn (1921) and Waksman (1919, 1920) has resulted only in the establishment of numerous species or strains which are unlike in only a few characteristics, some of which are not permanent in cultures. For this reason the writer has not attempted to name any of the actinomycetes found in soil or humus but has grouped them as non-color-producing (*Actinomyces albus*) or color-producing (*Act. chromogenus*).

THE PRESENCE OF SCAB LESIONS IN THE POTATO PLANT

On Stems and Stolons

The vertical stems of a large number of potato plants grown on infected soil were examined carefully for lenticels and for lesions

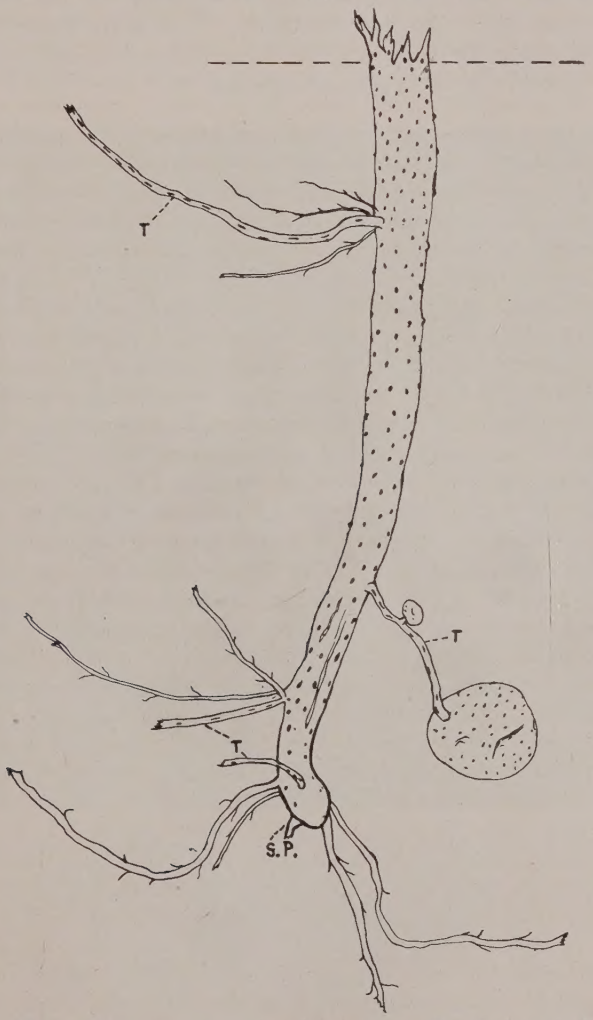

FIG. 1. Potato stem with roots, tuber stolons (T), and seed piece (S. P.) showing distribution of lenticels.

similar to those known as scabs on tubers. Tangential slices cut with a razor from such stems were mounted in 10 percent glycerine,

which was allowed to evaporate on the slides before the sections were covered and examined under the microscope.

The short, underground, vertical stems of potato plants are almost as well supplied with lenticels as are the tubers (Fig. 1), and the slender, white, horizontal stolons which attach the tubers to the mother plant also have lenticels. These stem lenticels, which are morphologically similar to those of the tubers, offer the same ready openings for actinomyces filaments.

FIG. 2. A. Stem, stolons, and roots of a potato plant grown in a light sandy soil. All the tubers on this plant were badly scabbed. The numerous dark brown lenticels on the stem and stolons show up against the white tissues. Two lenticels on the stem are much enlarged and browned. B. A root magnified eight times to show one of the dark-brown discolorations.

Deep infections were not common on these stems, although young tubers which had already formed on the plants were covered with small scab spots at their lenticels. A careful examination of the stems showed numerous clear-walled, nearly white lenticels and many dark-brown, slightly swollen ones. The casual observer may overlook the hypertrophy in these cases, since it is very small. This type

may be seen on the stem in Figure 2, A, infection in this case being general. These plants were grown on a rather dry, sandy loam soil in which the water content rarely exceeded 20 percent of the dry weight and was more usually about 15 percent. Under these dry conditions the organisms did not spread far from the lenticels.

Since these lenticels did not appear very distinctly in a photograph taken of the stem surface, tangential sections of the outer layers of the stems were mounted in glycerine and photographed.

FIG. 3. Tangential slices of the skin of potato stems; four lenticels in the upper slice are browned and swollen; three in the lower slice are slightly abnormal. 25×.

The upper of the two sections in Figure 3 shows five or six lenticels which are browned and slightly swollen, but the lower section is almost free from such infections. While these infected lenticels were dark brown in color, they were not markedly hypertrophied.

The vertical underground stem of the potato plant may become quite badly infected with typical scab actinomycetes although marked hypertrophy is not very common, possibly because there is no cork

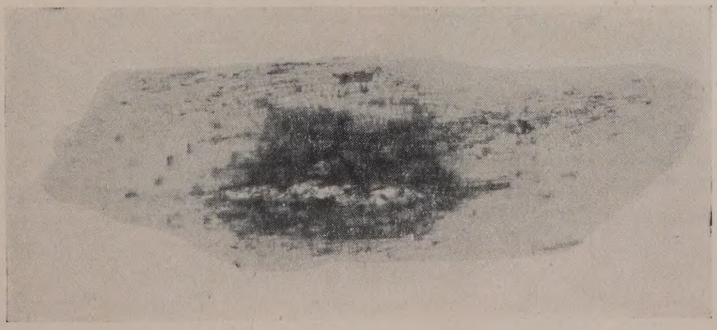

FIG. 4. Tangential slice of the skin of a potato stem, showing a single large, brown lenticel. 8×.

cambium to stimulate. These small lesions may be readily overlooked, as they have been in the past, but they are very different in character from the diffuse, black splotches which mark rhizoctonia infection or the diffuse, black, necrotic regions which Edson and Shapovalov (1918) attribute to various other soil fungi.

FIG. 5. Potato plant with large, light brown, cracked lesions on the large stem. The tubers from this plant were scabby.

The infection was found to have spread in a few cases to form a brown patch, such patches ranging from one-half to several square centimeters in area. Two of these larger infections appear on the stem in Figure 2, A. In a tangential section, Figure 4, the browning is seen to be diffuse and irregular. In some cases, a crack had developed at the center which opened into the deeper-placed tissues.

On the stem shown in Figure 5, the hypertrophy has progressed even farther. Large, light-brown welts which had cracked open were common on the stems from this lot of potatoes. These lesions extended to a short distance above the soil. The potato plants in this lot were grown on soil in which the water table was only about a foot below the soil surface and the stems were kept damp at all times, even during a very dry summer.

On plants growing in infected soil the stolons, some of which become modified and produce tubers, are covered with the same brown, enlarged lenticels (Fig. 2, A). On potato plants growing in dry soil, these lesions are very similar to the smaller ones found on the tubers, although they are more elongated because of the rapid growth in length of the stolons (Fig. 2, A). On plants grown in wetter soil, the lesions appear as elongated, light-brown splotches on the white stolons (Fig. 6).

No attempts were made to isolate pathogenic actinomycetes from these stems and stolons. The plants were grown in soil so badly infected with scab strains, that, even if any such isolations had been made, the objection could easily have been raised that they were obtained from the adhering soil and not from the browned tissues of the lenticels.

FIG. 6. Tuber stolons from a potato plant grown in moist soil. All tubers on this plant were scabby.

Exactly the same type of lesions may be found on the stems and stolons shown in Figures *c*, *d*, and *e* of Plate III in the bulletin by Jones, McKinney, and Fellows (1922) which has already been cited. These lesions appeared on potato plants grown from disinfected seed in sterilized soil which were inoculated with scab-producing organisms, but on uninoculated checks, the lenticels of stems and stolons were white.

On Roots

If potato roots can be infected by a scab-producing actinomyces, they may serve as an important factor in the perpetuation of such

organisms from year to year, since the root system of the potato plant is very extensive and all of it remains in the soil after the tubers are harvested.

Conn (1916) found over twice as many actinomycetes developing on plates made from sod soil as on those made from cultivated soil of the same type. More developed on plates made from old sod than on plates from sod only two to three years old. The explanation was suggested that the difference was due to the activity of actinomycetes in the decomposition of grass roots.

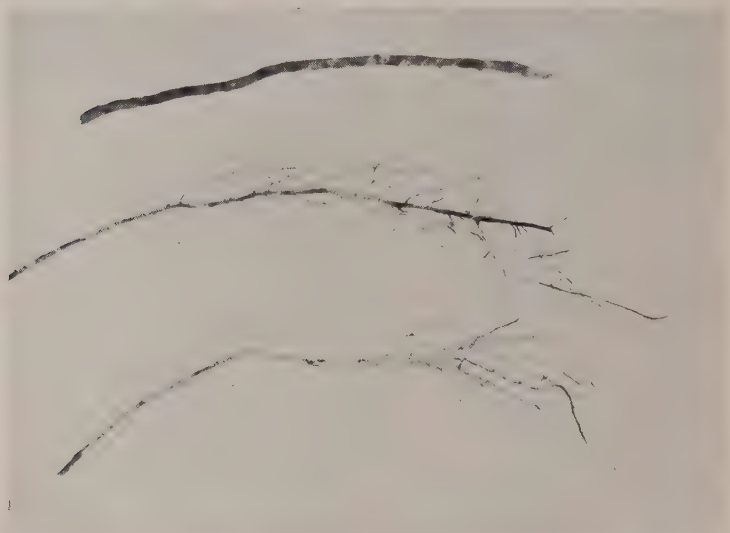

FIG. 7. Roots from another plant grown in moist soil. The large, old root at the top has much of its surface browned. The other two roots, especially the bottom one, are almost pure white.

A careful examination of potato roots indicates that, while they are normally white, brown splotches sometimes appear on them, especially as they age. Roots are not provided with a cork layer, as are the tubers, so that the browning must be the result of some growth between or on the walls of the epidermal or cortical layers.

Roots from plants which showed infection in their tubers, stems, and stolons are reproduced in Figure 2, A and B, and Figure 7. Figure 2 shows the roots of a potato plant grown on a fairly dry, sandy loam. The larger roots were white with light-brown lesions. The young roots were entirely white. In A, the roots are reproduced normal size; in B, a root is magnified about eight times so that the

general nature of these brown areas may be seen. Their resemblance to the diffuse lesions on the stem is very marked.

The three roots shown in Figure 7, from plants grown in a very moist soil, are reproduced about natural size. The old, large root at the top was light brown in color but was blotched with darker-brown patches. The two younger, fibrous roots were almost white except for a few small brown patches and the browned terminal ends of several rootlets.

The cause of the browning of roots is not as evident as the cause of the pronounced lesions and browning of the stems and stolons, because roots are not provided with lenticels where deep scabs originate. The only way in which the location of the irregularly diffused material can be determined is by examining sections of roots under the microscope.

Thin tangential sections of the cortex and cross sections of the entire root were mounted in glycerine and examined under the microscope.

The tangential sections show that some of the cell walls are browned. Figure 8, A and B, show two unstained sections from such a region. In A, some of the walls, whether in or out of focus, appear white, and others are transparent. However, two or three walls which are in sharp focus are almost black. The multiple twisting and branching of these dark walls should be noted. The blackness and sharpness of the walls and the unstained, adjacent cytoplasm inside the cells should be also noted. A somewhat different condition, which is probably a more advanced browning, may be seen in B of this figure. Here a large, dark-brown area on the right is too dark to show details clearly, but near it the walls of three or four cells, together with their cytoplasm, are in sharp focus. The walls are not thin, straight lines but are wide and twisted, in some places being light and in other places dark, whether in or out of focus. In the adjacent cytoplasm, dark and light areas alternate, due apparently to excretion of the stained material from the cell walls.

To check these longitudinal sections, cross sections, made with a razor, were examined in unstained glycerine mounts (Fig. 8, C and D). C, which has a low magnification, shows a cross section of the cortical cell walls in a root which was about the diameter of a coarse needle but had a cortex composed of only four or five cell layers. The browning is restricted to the walls of the outer layers. The walls seen here are very complex structures and are not single lines, as they are usually made in root drawings. The walls of the outer layer, which were pulled out of position by the razor in cutting, show details of structure.

In D, a small part of the upper left-hand corner of C is reproduced at a slightly different focus and with a higher magnification. The complexity of the strands makes the root look as though it were provided with a very elaborate system of mycorrhizal hyphae im-

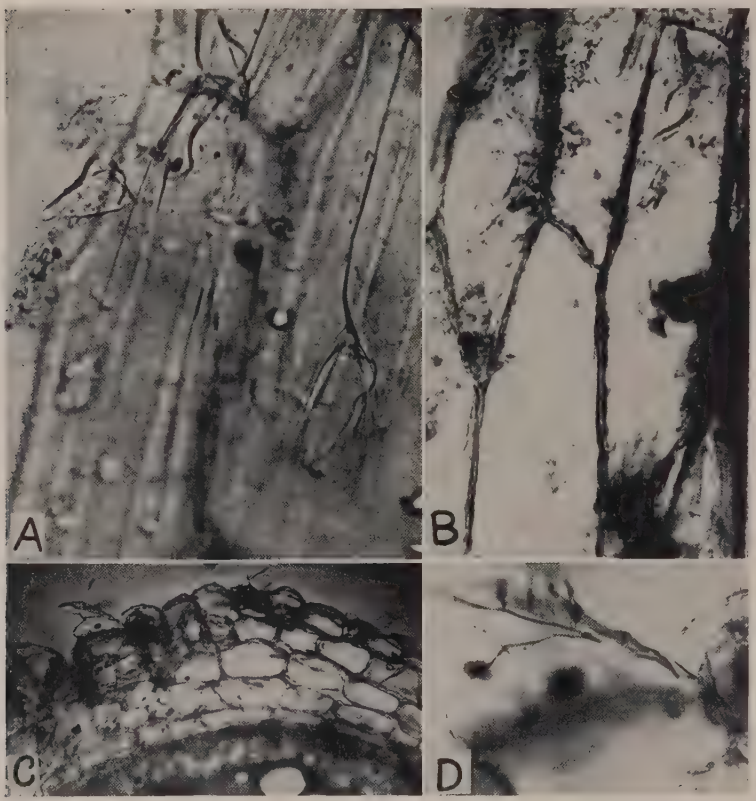

FIG. 8. Unstained sections of brown potato roots from infected plants. A and B are tangential. A, dark strands simulating cell walls are sharply focussed and their branching and twisting may be traced. In B, a dark-brown region has been largely trimmed away at the right but the swollen and dark-brown walls of the epidermal cells to the left are in sharp focus and areas where dark color has been excreted into the cytoplasm can be seen. C, a cross-section of a small potato root, shows the complicated structure of the walls of the cortical cells. Some of the walls are browned and one at the left has been pulled out of position. In D, this region has been highly magnified to show the complicated structure of the wall. A, B, and D, 700×; C, 150×.

bedded in its cell walls. The twisting and branching indicate that these stands are filamentous, rather than lamellar. The writer believes them to be filaments of an actinomyces. They could not be Mangin's middle lamellae since his theory could not account for the excretion

of brown pigments into the cellulose walls and the adjacent cytoplasm, and such excretions certainly occur.

Unfortunately, in the illustrations of infected stems in the paper of Jones, McKinney, and Fellows (1922), the roots do not appear, since they had been carefully removed, and no mention of them is made in the text. However, the short stubs of roots which were left on the check stem are as white as the stem itself.

The evidence offered by Beijerinck (1900, 1911, 1913) that the underground parts of a number of species of plants, mostly trees and shrubs but also one herbaceous species, are infected by actinomycetes cannot be ignored, for Beijerinck was not only a very competent microscopist and soil microbiologist, but also an excellent plant anatomist.

The nature of this brown pigment must be left undecided. In 1900, Beijerinck was positive that the pigment produced by the action of *Act. chromogenus* on peptone was chinon but in 1913 he was as decided that the actinomyces which infested elm roots produced melanin from tyrosine, but only when in symbiosis with another root bacterium. The whole matter of color production by actinomycetes needs reinvestigation. Afanasiev (1937) has reopened the subject. Any such investigation, however, will at once encounter some difficulties: 1. The chemical composition of melanin is unknown and seems to vary; 2. No chemist is certain as to the purity of his melanin; 3. Natural melanin may be different from that obtained from tyrosine; 4. No test is known for small amounts; 5. Chemists are uncertain whether there exists one or many melanins. On account of their presence in skin and eye pigmentation and in certain types of cancer, they have been studied intensively by medical biochemists (Percival and Stewart, 1930).

A Differential Medium for Actinomyces Culture

So far as the author knows, no previous investigator has suggested a differential medium for growing soil actinomycetes. The rapid and profuse growth of pure cultures from the soil in a combination of pectin and inorganic salts suggested that a similar combination might cause these organisms to germinate. A number of trials using the gum tragacanth medium were not successful. The same medium made without gum tragacanth is a liquid of about the consistency of ordinary mucilage. Organisms from soil smears made a good growth on this liquid medium.

Liquid Pectin Medium

The following procedure was used. One gram of the soil was shaken in 10 cc. of a 0.15 percent solution of gelatin. The coarser particles were allowed to settle out before enough of the upper layer was removed with a capillary pipette to make a small droplet, 2 to 3 millimeters in diameter, on a cover glass. After a period of air drying, this smear was covered with a small drop of a liquid which was made up of 1,000 cc. of tap water, 10 grams gum arabic, 2 grams l-arabinose, 2 grams K_2HPO_4, 4 grams NH_4NO_3, 0.5 gram $MgSO_4$, and 2.5 grams $CaCO_3$. This pectin medium had a pH of 6.2. It was so cloudy that centrifuging was necessary to clear it, after which it was poured into small flasks, stoppered with cotton, and sterilized. The necessary amount could be removed with a pipette and the remainder boiled for further use.

The cover glass, with the small dried smear of soil covered by a drop of this medium, was then inverted on a slide, thus forming a hanging drop culture, and the slide placed in a damp chamber. Germination occurred in about 24 hours.

After the pectin medium had been carefully washed off with a slow stream of water, the smear could be stained with a warm rose bengal solution over a water bath. While many actinomyces filaments would wash off in the operation, many others remained. However, nothing could be seen as to the origin of the filaments that could not be made out in unstained culture. The hyphae could be traced back into the bits of humus but their point of origin was usually concealed by the granular composition of the latter, further aggravated in many cases by its dark-brown color. No conidia nor chains of conidia were ever observed nor did germinating hyphae ever come from an organ which could be interpreted as a spore in the stricter sense in which this term is used.

Liquid cultures presented some difficulties. The bits of humus had a tendency to settle out on the periphery of the drop, along with fine sand granules of one to three microns diameter. The germinations were difficult to photograph because of the uneven distribution of the organisms and the fact that they were suspended in a liquid. In later experiments the same medium was used, but in the form of a gel, one-half percent agar being added.

Gelled Pectin Medium

The gelled pectin medium was devised to determine the presence, as well as the life cycle, of the pectinase-forming types of the soil actinomycetes, among which are the potato scab organisms, by growing them directly from the soil. It was very successful with a light

sandy loam soil, and has also been found successful with other field and garden soils during the summer months. The procedure is given here in detail. Bacteriologists will recognize parts of Conn's staining method for soil bacteria and Frost's little-plate method for milk bacteria.

1. Thoroughly shake one gram of soil in 30 cc. of a 0.15 percent gelatin solution and then allow it to settle for five minutes. A slender graduated cylinder was found most useful for this work.

2. Insert a Breed capillary, $\frac{1}{100}$ cc. pipette about two inches into the upper layers of the liquid, keeping the finger on the pipette end during insection. Suck up a little of the liquid and remove the pipette. Wipe off the lower end of the pipette with a clean cloth before placing droplets of the liquid (2 to 4 mm. in diameter) on clean covers to dry.

3. Prepare the culture medium as follows:

Tap water	1,000 cc.	NH_4NO_3	4.0 grams
Gum arabic	30 grams	$MgSO_4$	0.5 gram
Arabinose	2 grams	$CaCO_3$	1.0 gram
K_2HPO_4	2 grams		

Boil, to dissolve the gum arabic. Centrifuge to clear, and then add 0.5 gram of agar (0.5 percent) and boil. Sterilize and keep in small flasks until used.

The pH of this solution is 6.4, but on standing a few days it changes to 6.0 and after a week or so becomes about 5.6. The centrifuging removes part of the excess lime but the remainder is held in the colloidal gel in a finely divided state.

4. Liquefy the culture medium by boiling. Cool. With an ordinary 1 cc. sterile pipette, cover the dried spots on the cover glasses with small drops of the culture medium. Invert cover glasses and seal with vaseline to deep-chamber culture slides.

5. Incubate at room temperature for from 15 to 24 hours. The actinomyces filaments (one to numerous germ tubes) may then be seen emerging from the soil humus fragments.

The liquid medium used in the first summer's trials contained no agar. Even the purest commercial agar still retains various nitrogenous compounds and inorganic salts. Germination and growth in this liquid medium containing no agar were excellent. The only organic compounds in it (other than the pectins) were from the trace of gelatin used in the dilution (0.15 percent) and this amount could not have accounted for the continuous growth of the actinomyces hyphae.

The gelatinous medium used in the later trials was solidified with 0.5 percent of agar and the organisms could obtain some organic nitrogenous food from this source. In fact, some germination could be

obtained by covering the small smear with the 0.5 percent agar solution alone.

The combination as finally used was fairly acid (pH 5.6). This is much more acid than any medium used for bacteria but not as acid as the medium which gives optimum growth with the majority of the molds and yeasts, which has a pH of 4.8.

Only a small percentage of the bacteria can make use of the pectin compounds contained in this medium, or of arabinose. While yeasts and molds will grow in this medium, they are largely removed when the solution is allowed to settle for five minutes. An occasional yeast or mold will be held in one place by the agar and will form a colony, but usually this will not interfere with observation of the actinomycetes if the preparation is examined within 12 to 24 hours. The cultures have to be examined at intervals since germination varies with the previous history of the soil, its temperature, and its water content.

If these precautions are followed, this medium and technic may be termed differential. In order to make use of the pectins, an organism must secrete pectinase and the medium is differential in this respect.

THE GERMINATION OF ACTINOMYCETES FROM SOIL AND POTATO HUMUS

Germinations from Soil and Disintegrated Humus

In 1942 and 1943, germinations were made largely from potato plant humus which had been in the soil from two to eight years. It was so thoroughly disintegrated that few structures resembling plant organs could be traced. The bits of humus were simply granular, colloidal, brown or colorless, amorphic, microscopic masses of varying sizes. Humus bits still containing cells or walls could occasionally be found but they were quite rare.

The humus particles removed were from 10 to 70 microns in size, the majority being between 20 and 30 microns. Best results will be obtained when there are from 10 to 20 particles visible in the high power field. With this concentration, the individuals will be sufficiently separated so that the emerging hyphae may be traced.

By this method one can make an approximate count of the number of actinomycetes in a soil which will grow on this medium. If the dilution, settling time, depth to which the pipette is inserted, and size and number of particles are kept constant, it should be possible to make a comparative count.

Before discussing the results obtained, it is necessary to recall some facts with regard to the terminology used. Two phases are

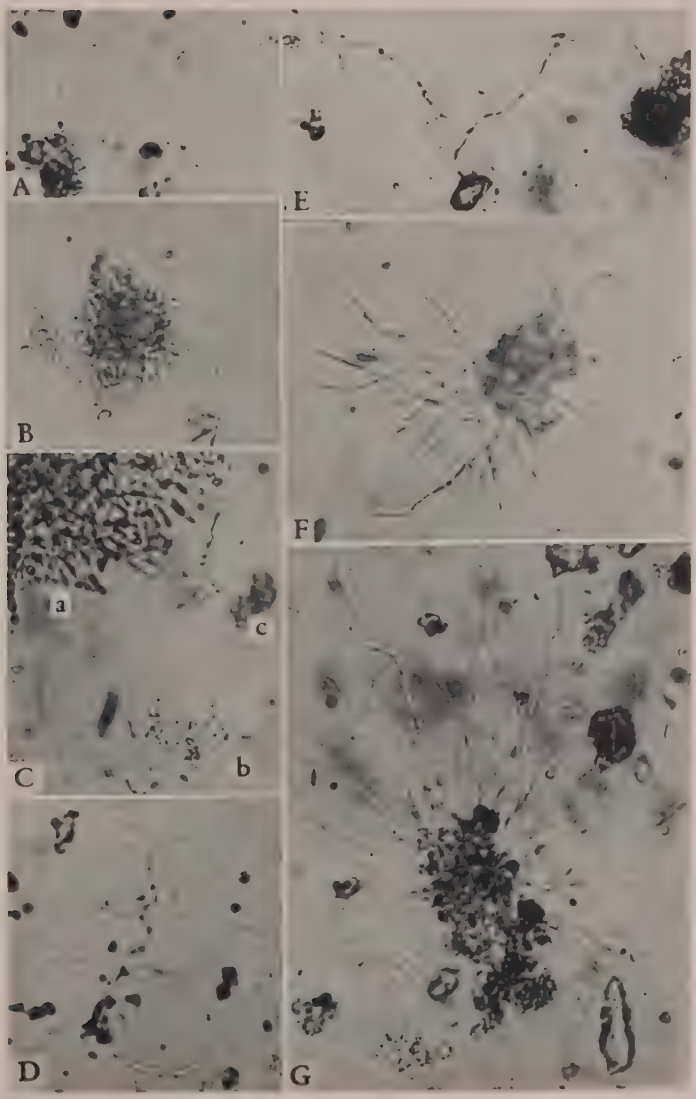

FIG. 9. Actinomyces hyphae emerging from bits of old potato humus in smears covered with pectin medium, showing the varying number of hyphae which may come from one piece of humus. The impossibility of determining the source of the hyphae inside the granular particles is apparent. C is of interest since it contains, in one field, a mold colony (a), a small bacterial colony (b), and actinomyces hyphae emerging from a bit of humus (c). 700×.

usually described in the life cycle of the actinomycetes, vegetative mycelium and conidia or spores, but between these extremes, the actinomycetes exhibit a wide range of intermediate resting fragments of mycelium. Any fragment of the mycelium may serve as a spore and the ability of such a fragment to germinate will depend upon the previous treatment (cold, drought, lack of food, etc.) it has received.

The difficulties will be apparent from an examination of the germinations shown in Figures 9 and 10. The most transparent examples are D and E of Figure 9 and A, B, D, E, and H of Figure 10. A careful examination of Figure 9, D, reveals that germination occurred at both ends of a piece of hypha fastened to the dark bit of humus in the lower center. The upper hypha is much longer than the lower one. In E, of the same plate, a bit of hypha seems to be attached to the humus particle and to have also germinated at both ends.

Figure 10, A, shows the origin of the hyphae at the side of a very small humus bit. The nature of the point of origin, whether a spore or a short piece of hypha, is not clear. This is true also of B and H, although in H, the point of origin may be an oval spore. In Figure 10, D, the two hyphae can be traced to a clear bit of mycelium imbedded in the light-brown humus.

In Figure 10, J, the germ tube may have originated from a spore. This germination occurred in a culture made from a soil sample which, after being shaken with 30 cc. of the gelatin solution, was allowed to stand and settle for 25 minutes instead of the usual 5 minutes, with the result that all particles which were removed by the pipette were only a few microns in diameter. The hyphae seemed to emerge from an oval body which may have been a conidium.

One fact should be noted about the germinations from bits of humus. While some filaments came from quite transparent humus particles (Fig. 9, B), the majority came from dark-tinted ones (Fig. 9, F and G, and Fig. 10, C, D, F, and K). In any case, however, the granular composition of the humus makes any definite conclusion regarding the location and nature of the spore or bit of hypha from which the growing hyphae originate quite impossible. Even the number of sources cannot be ascertained, since many of the emerging hyphae branch repeatedly (Fig. 9, F and G). While a single hypha may grow out of one humus particle, as many as fifty hyphae may come from a larger bit.

Figure 10, G, shows the only case found of a hypha coming from a bit of hypha attached to a quartz fragment. F and L of the same plate appear to be still recognizable plant remains with a number of hyphae coming from them.

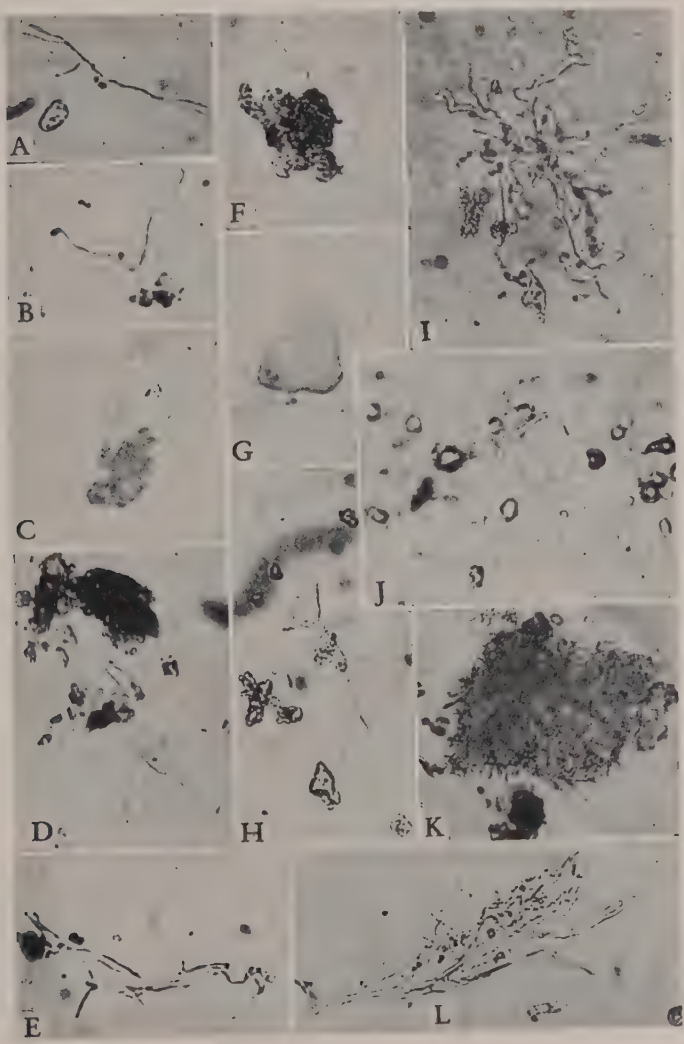

FIG. 10. Similar series of actinomyces germinations, showing further variations. G shows one of the few cases seen in which hyphae came from a bit of mycelium attached to a quartz fragment. H and I show the same hyphae taken 12 hours apart. In K, numerous short hyphae are seen emerging from a large, dark bit of humus. This preparation was left on the microscope stage for 12 hours, but none of the hyphae lengthened, indicating that this medium is not favorable for the growth of all soil actinomycetes. The same checking of growth was observed in E and L. 700×.

As previously stated, this pectin medium will support the growth of molds, yeasts, and a few bacteria as well as of actinomycetes. By a happy chance, three of these groups were obtained in one field in Figure 9, C. A mold (or mycoderma) colony occupies the upper left hand corner (a), the actinomyces is seen emerging from a piece of humus in the middle right, (c), and a small colony of bacteria is found at the bottom of the photomicrograph (b).

H and I of Figure 10 both show the same germination. H was taken at about 4 P.M., after which the culture was left on the microscope stage. By the following morning, the hyphae had branched and lengthened into a very complicated tangle, as seen in I which was photographed at about 9 A.M.

On the other hand, occasional germinations, such as those seen in Figure 10, E, K, and L, would show numerous short hyphae but even when the slides were left for 24 hours no further growth would take place. The medium in these cases seemed to be adequate to start growth, but not to maintain it. The medium may have contained enough soluble material, in addition to that in the humus, to induce a slight growth but some enzyme (probably pectinase) which would have made pectins available was lacking or too weak to provide further growth.

With regard to this question of continued growth, as distinguished from the short hyphae obtained in germination, the following may be said about this pectin medium. Excellent growths of isolations from soil and humus of both *Actinomyces chromogenus* and *Act. albus* were obtained in stab cultures of this medium in test tubes after incubation of from 4 to 7 days at 37 degrees C. In general, conidia formation and growth of mycelium were as profuse as those obtained on transfers to the ordinary nutrient agar, although they were not as large as those obtained on the gum tragacanth combination.

Starkey (1938) found the soil stage of the actinomyces group to include not only mycelial growth but also conidial formation and germination. The discrepancy between his results and those described in this paper must be due to the methods used. Which method gives the true picture of activity and dormancy of this important soil group? Starkey inserted a glass slide vertically into the soil. Advocates of this technic claim that soil organisms which come in contact with the slide continue their growth and activity undisturbed and some of them will be found on the stained slide just as they were in the soil. The writer would like to point out that any such soil-cut disturbs the soil-particle arrangement, and no amount of care can obviate this. New air pockets are introduced and when a slide is inserted these pockets remain between it and the soil surface. Rape,

vetch, and other plants had been grown on this soil and their roots would have further disturbed the soil-particle arrangement as they pushed their way through it.

The aerial mycelium of the actinomyces fragments into conidia as it dries in these pockets, just as it does on a scab culture surface, in spite of the humidity of the soil or of the incubator. In general, spore formation is simply a process of dehydration. This is especially true in the case of the bits of protoplasm generally known as conidia, which are formed by fragmentation inside of a sheath. When the slide is removed from the soil and stained, pieces of mycelium and chains of conidia are found on it, usually, as Starkey has suggested, in the vicinity of decomposing organic matter. Root excretions when plants are grown in the soil may also supply the requisite minimal requirements for germination and growth.

The Cholodny method cannot give much information on the origin of filaments. The pectin medium technic described by the writer does enable the observer to follow the filaments into the bits of humus. The latter may be too granular for accurate observations in most cases, but in the more transparent humus particles, the filaments appear to come from a short piece of hypha. The distinction between these pieces of hyphae and the so-called conidia is not sharp and the two classes merge into "resting bodies."

Germinations from One-Year-Old Potato Humus

The germinations of soil actinomycetes which were carried out in the summers of 1942 and 1943 from old humus in the soil were so successful that attempts were made in 1943 to obtain germinations from plant remains from a humus bed that had been made the preceding fall. These potato tops had been covered in masses in a green condition and the leaf veins and laminae and, of course, the larger stems and leaf petioles were still intact in July, when the first trials were attempted.*

In the soil cultures a dilution of 1 gram of soil in 30 cubic centimeters of gelatin solution had been used but with the humus dilutions of 1 to 1,000 were necessary. The humus contained 70 percent water at the time of the experiments (July 16) while the adjacent soil had less than 7 percent.

The humus was triturated thoroughly in a mortar but, of course, some groups of plant cells could still be found in a recognizable condition.

* The author was unable to find any data on the persistence of humus in the soil in this climate. In the tropics all humus burns out of the soil in a few months and one trained observer expressed the opinion to the writer that humus persists for one season only in the warmer regions of North America.

Germinations were even better than those obtained from old humus in the soil. Some typical bits of humus with the actinomyces threads issuing from them are reproduced in Figure 11. All of these fragments are of leaf origin, with the possible exception of D.

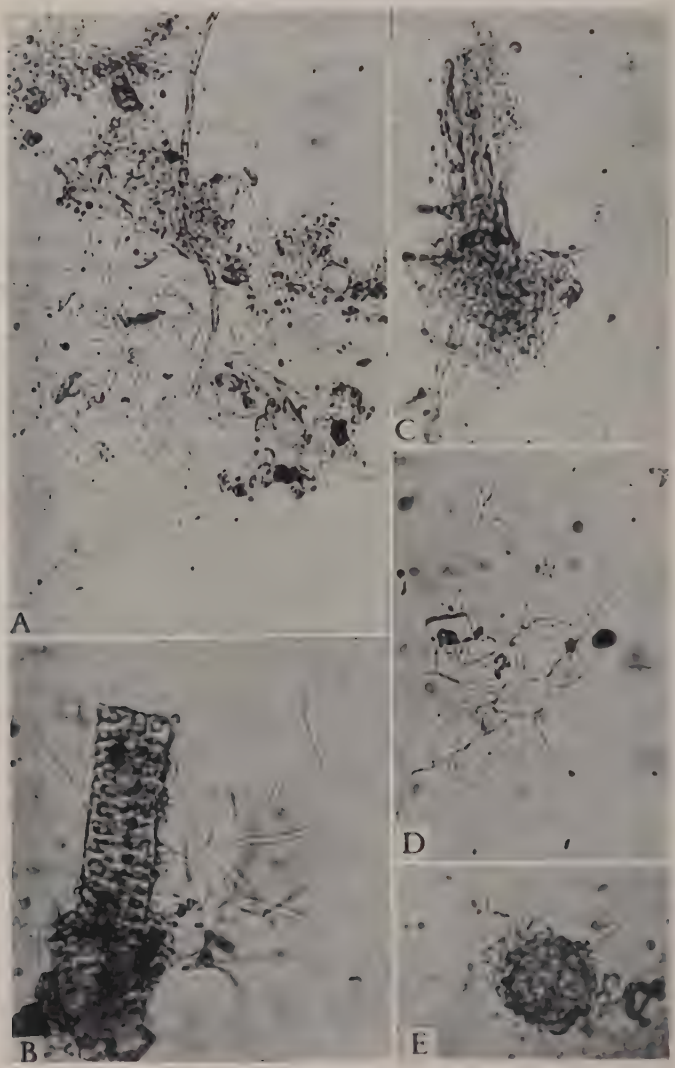

FIG. 11. Growth of actinomyces filaments from finely ground potato humus from a humus bed made the preceding year. This humus was principally leaves and large stems; plant structures could still be recognized. Note variation in the diameter of hyphae arising from various organs. 700×.

Figure 11, A, shows only a part of the fragment photographed, which was a leaf lamina; C and D were smaller pieces of leaf laminae, while E was the base of one of the leaf hairs. Figure B was a large vessel, broken from a leaf petiole or a small stem.

No comments are necessary on these figures other than to call attention to the variation in the diameter of the actinomyces filaments. All photographs were made with the same lens combinations but the filaments in A are very delicate as compared to the filaments in the other figures. Whether this difference in size indicates different species cannot be said; some investigators lay considerable stress on filament diameter as a species characteristic (Duché, 1934). In this connection, it should be stated that the bacteriological plates gave as high as 16,000,000 colonies of the *Actinomyces albus* group per gram of the same humus and all of the colonies seemed to be the same species. No further attempts to differentiate species were made.

These germinations of actinomycetes from humus were made at least three times in 1943 and were successfully repeated from the humus beds in the summer of 1944.

THE RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE GERMINATION OF ACTINOMYCETES

In 1942 all efforts to obtain germination during May and early June were without success and it was not until nearly the middle of July, 1942, that a medium and technic were found which induced germination. During the rest of July results were very successful. The germinations were most numerous about the first and second week in August. They were still abundant the first week in September. The experiments were not continued beyond that date.

The following summer (1943), trials using the same medium and the same soil were begun May 17, in the expectation that studies on the germinations could be continued. No germinations occurred. Attempts on newly made media were unsuccessful. The recipe was repeatedly changed but without results except that, in the experimentation, it was found more convenient to use a medium in which only a small percent of agar was added to make a weak gel. Then, on June 12, some germinations appeared in the cultures. The number of germinations kept increasing until July 14. At this time, the writer recalled the very significant work of Jones, McKinney, and Fellows (1922) on the effect of temperature on potato scab infections. Various factors might be involved in scab infections but in any case the soil temperature would have to be high enough to permit gemination of the scab-producing actinomycetes.

Very fortunately, soil temperatures were available, which had

been taken by Dr. J. W. Marvin on another field of this farm during the growing season of 1943. These soil temperatures are presented through his courtesy. These temperatures were taken at a depth of between two and three inches, which is about the same depth as that at which the soil samples used for the germination studies were taken.

Date	Temperature *Degrees C.*	Date	Temperature *Degrees C.*	Date	Temperature *Degrees C.*
May 17	9.5	July 26	22	Sept. 27	16
" 24	14	Aug. 2	22	Oct. 4	15
" 31	15.5	" 9	22	" 11	13
June 7	18	" 16	22	" 18	13
" 14	16	" 23	21	" 25	11
" 21	19.5	" 30	21	Nov. 1	10
" 28	19.5	Sept. 6	20	" 8	10
July 5	19.5	" 13	20	" 15	7
" 12	23	" 20	19	" 22	4
" 19	24				

The soil temperature was about 18 degrees C. when germinations first began in June, although the optimum for scab infection was found to be about 22 degrees C. under the controlled tank conditions used by Jones, McKinney, and Fellows. Scab infection involves two factors, the growth of the potato plant and that of the infecting organisms. In this work, the author was dealing with the latter factor and ignoring the former.

On July 11, when the germinations had become very numerous, the soil temperature was about 22 degrees C. But on July 14, soil samples brought into the laboratory gave no germinations after the usual 24-hour incubation and only a very few on some slides after 48 hours, although more appeared after 72 hours. Fortunately the yeasts and molds were similarly retarded in their growth. A few days of warm, dry weather had reduced the moisture content of the soil from 16 percent to 6 percent of the dry weight.

On July 23, although there had been a good rain and the soil contained 12 percent moisture, the germinations were about as poor as before. The rain had not stimulated growth. Results of trials made on August 23 were slightly improved but even so the germinations were no better than those obtained at the beginning of the season on June 12. On September 22, when the ground contained 20 percent water, the pectin-medium cultures showed rapid growth and numerous long hyphae in 24 hours. Growth was almost as good as that obtained in early July. The cultures grew almost as rapidly and as vigorously on October 28 as in June, although the temperature of the soil had now fallen to 10 degrees C. with a moisture content of 22 percent. The cultures made on this date were the last for the year.

In 1944, pectin-medium cultures were first made on samples taken on May 18. A few germinations could be found coming from the bits of humus after 48 hours. The soil had been warm but dry during

early May of that year. The germinations were still few in number on June 16 and 28. On July 12, although the soil sample held only 6 percent water, germinations continued numerous and hyphal growth rapid. The germinations were as numerous as any seen the previous summer, in spite of the low water content of the soil, on July 17. On July 25, pectin cultures made from quite dry soil (containing only 9 percent moisture) gave numerous hyphae in 48 hours. The dry soil seemed to delay germination in spite of the favorable soil temperature, so that 48 hours were required instead of the usual 24 hours. Germinations were rapid and numerous on a trial made in August.

The last attempt made to germinate actinomycetes from bits of humus was made on soil samples which were brought into the laboratory on December 9 and kept in a cool room until December 11, when the pectin cultures were made. No germinations were seen on December 12, but on December 13, one or two germinations could be found on each culture. The soil temperature at this date had fallen to +0.5 degree C. The germinations of molds and yeasts were also very slow and few in number.

In their controlled experiments on scab, Jones, McKinney, and Fellows provided a constant moisture content of 18 percent for the soil in the tanks and varied the temperature. The principal effect of low soil moisture content seems to be to delay germinations so that 48 or 72 hours are required instead of the usual 24 hours. These summer germinations and growth rates are the result of a combination of two soil factors, moisture and temperature. With scab infections a third factor is introduced, the growth of the young potato tubers.

Goss (1937) found that under Nebraska conditions scab was usually more severe in the fields in seasons of high rainfall than in dry years and that the scabs occurring when the soil had a high moisture content were severe and often pitted.

Goss' work was done in a region where the rainfall was usually too small to permit maximum potato yields. On the other hand, Sanford (1926, 1945) working in Alberta, noted that soil which was either too dry or too wet produced less scabby potatoes than soil which contained the optimum moisture content for growth. In other words, a soil with an optimum water content for potato growth and yield would be likely to show also an unusually high percentage of badly scabbed tubers.

These temperature and moisture factors would seem to regulate the severity of scab attacks on sandy, light land, a wet growing season with moderately warm temperatures favoring germination of the actinomycetes and giving them an opportunity to effect an entrance

into the young tubers. Once the intruders are inside the potato, fluctuations of moisture and temperature would not be felt by them to any extent. The depth of the scabs would depend upon the frequency of rainfalls, each rain offering an opportunity for a deeper penetration by the actinomycetes into the growing tuber.

The 1943 season was ideal for scab infections on light land and on early-planted potatoes. When the plant shown in Figure 2 was brought in during July 1943, all the tubers were scabbed.

THE PERSISTENCE OF ACTINOMYCETES IN THE SOIL

In September 1936, soil from the plot which was in use for the scab experiments was brought into the laboratory and mixed with 10, 20, 30, and 40 percent (by weight) of a fine, black, forest leaf mold, and 20 percent water was added to each mixture. The soil itself contained about 3 to 4 percent of humus by weight. These mixtures of soil and humus, as well as some samples of soil alone, were placed in a series of mushroom-stoppered glass bottles of about 200 cc. capacity and autoclaved.

A series of cultures of strains of *Act. chromogenus* were incubated to obtain maximum growth. The mycelium and conidia were then removed to mortars containing a little sterile sand and water and were ground up. This material was suspended in sterile water and an equal amount worked into the soil-humus mixture in each of the bottles. The glass stoppers were replaced and the bottles were placed on a laboratory shelf and not opened for two years.

In September 1938, samples were removed from the bottles to determine the amount of humus still remaining, and the number of organisms present. The results are given in Table 1.

Table 1. Percentages of Organic Matter and Numbers of Organisms Present in Mixtures of Soil and Humus Bottled in September 1936

Determination	Soil alone	Soil mixed with 10% humus	20% humus	30% humus	40% humus
Determinations made in 1938					
		Percent			
Organic matter	3	8	10	13	27
		Millions			
Total organisms	***	95	147	32	***
Act. chromogenus	***	83	128	18	***
Determinations made in 1939					
		Millions			
Act. chromogenus	134	31	277	19	***

* Not plated.

In 1943, four bottles were opened and samples plated on nutrient agar. These bottles had been opened before, at which time no special precautions had been taken against contamination from the air, but in spite of this, the actinomycetes were still very abundant. The numbers of *Act. chromogenus* present in the four bottles were as follows:

Bottle 1, soil with 10 percent humus	16,000,000
Bottle 2, soil with 10 percent humus (a nearly pure culture)	5,000,000
Bottle 3, soil with 20 percent humus	187,000,000
Bottle 4, soil with 20 percent humus	470,000,000

No special precautions were taken to avoid external contamination. The bottles were replaced on the shelf. The following year, samples were taken from two bottles and again plated. The glass stoppers of some of the bottles did not fit accurately, and after six or seven years, the moisture in these bottles had fallen so low that organisms could no longer exist.

In the two bottles from which plates were made in 1944, the numbers of *Act. chromogenus* found were:

Bottle 1, soil alone	6,300,000
Bottle 2, soil with 10 percent humus	6,200,000

Dilutions from some bottles which contained numerous viable actinomycetes were stained on slides using the Conn technic and recognizable fragments of actinomyces filaments could be seen under the microscope.

Attempts to induce these fragments to grow in the pectin medium which was successfully used on soil from the field were entirely negative, no growth appearing even after a week. The fragments evidently had become so weakened that they could not grow under these conditions although they retained sufficient vigor to do so in the more readily assimilable constituents of the nutrient agar.

This experiment proves that soil actinomycetes are able to survive in large numbers in the soil for not less than seven years and probably for as long as the moisture conditions are favorable and the soil contains humus.

Some Characteristics of Potato and Buckwheat Humus

The potato humus was made without the addition of lime or other chemicals and the stalks and many of the leaves were still green when they were covered with soil in 1942. In 1943, the tops had remained on the field for about three weeks and were much drier except where they had accidentally been covered by soil. In both years the plants had been covered with Bordeaux mixture and some of it remained on the foliage.

Buckwheat humus beds were also made for comparison with the

potato humus. Buckwheat and winter rye are frequently used as cover crops to be turned under green. They are generally believed to have no effect on potato scabbing, or to lessen the severity of the attack.

Alkalinity

Samples from the 1942 potato and buckwheat humus piles, together with some of the soil used in the piles, were brought into the laboratory at various times during the summer of 1943.

Table 2. Acidity or Alkalinity of Soil and of Buckwheat and Potato Humus on Five Dates During the 1943 Growing Season

Material	Acidity or alkalinity, as expressed as pH, on				
	May 30	June 8	July 26	Aug. 23	Sept. 21
Soil	6.4	6.4	6.4	6.4	. .
Buckwheat humus	6.8	6.8	6.6	6.8	.....*
Potato humus	7.2	.....	7.2	7.2	. . .
Stems	.....	7.0	.....	.....	6.9
Leaves	.....	7.2	.....	.....	7.0

* All the buckwheat humus had decomposed by Sept. 21.

The figures in Table 2 show that potato humus persists in the soil and that it is markedly alkaline to the end of the growing season.

As previously noted, the potato plants had been kept covered with Bordeaux mixture and, of course, some lime was retained on the potato tops when they were placed in the humus pile. The alkalinity continued throughout the growing season, however, and most of this lime must have been removed by the heavy rains which characterized this season. The potato stems (to which little lime would have adhered) were as alkaline as the leaves. Moreover, the buckwheat humus also was more alkaline than the adjacent soil and the buckwheat had not been sprayed.

Moisture Content

The moisture content of the soil and humus samples was ascertained on a dry weight basis (Table 3). The potato humus held at

Table 3. Moisture Content of Soil and of Buckwheat and Potato Humus on Four Dates During the 1943 Growing Season

Note—Moisture content determined on a dry weight basis.

Material	Moisture content			
	May 20	July 26	Aug. 23	Sept. 21
	P e r c e n t			
Soil	20.5	19	20	20
Buckwheat humus	50	42	92	.....*
Potato humus	60	68	96	.....
Stems	.....	.....	.....	146
Leaves	.....	. . .	. . .	67

* All the buckwheat humus had decomposed by Sept. 21.

all times very much more moisture than the adjacent soil; in fact, it seemed to be always moist, even while the adjacent soil was comparatively dry. The spongy structure of the undisintegrated humus retained moisture, while rain percolated rapidly through the sandy loam soil and was lost.

Microorganisms

Platings of the soil and of the potato and buckwheat humus were made during the summer and fall of 1943 to determine which groups of organisms were predominant. No attempt was made to identify the organisms other than by groups, and the actinomycetes were divided simply into the *Actinomyces chromogenus* and *Act. albus* groups. The platings were made on nutrient agar and the counts were taken in about seven days. The results are given in Table 4.

Table 4. Number and Type of Organisms Present in Soil and in Buckwheat and Potato Humus on Four Dates During the 1943 Growing Season

Material	Organisms present				
	Total bacteria	*Actinomyces albus*	*Actinomyces chromogenus*	Molds	Yeasts
May 21					
Soil between humus beds	7,200,000	0	1,550,000	0	0
Soil shaken from humus	309,000,000	3,000,000	2,000,000	0	0
Potato humus, coarse	1,230,000,000	800,000	0	0	0
Potato humus, fine	950,000,000	6,000,000	0	0	0
Buckwheat humus, coarse	1,050,000,000	0	0	0	0
July 20					
Soil near humus beds	6,300,000	2,100,000*		0	0
Buckwheat humus	150,000,000	1,000,000	0	7,000,000	0
Potato humus	166,000,000	16,000,000	0	45,000,000	0
August 6					
Soil near humus beds	23,800,000	600,000	1,900,000	0	0
Buckwheat humus	439,000,000	69,000,000	2,000,000	3,200,000	103,000,000
Potato humus	414,000,000	59,000,000	1,000,000	21,000,000	68,000,000
October 29					
Soil near humus beds	14,700,000	1,000,000*		2,300,000	0
Potato humus	390,000,000	46,000,000	4,000,000	39,000,000	430,000

* *Act. albus* and *Act. chromogenus* combined.

The outstanding characteristic of these platings is the predominance of *Act. chromogenus* in the soil and of *Act. albus* in the potato and buckwheat humuses. In the May 21 platings, the two groups were nearly equally divided in the soil which was removed from the humus. In this case, the *Act. albus* group was probably largely derived from bits of the humus which found their way into the soil surrounding them. In this trial, the humus, which was largely stems, was washed under the tap, an operation which removed much of the softer, outer parts where the microorganisms, especially

the actinomycetes, were very abundant. In the later trials, the soil was removed from the humus by shaking it out dry.

The dilutions for making soil plates were always maintained at 1 to 1,000,000 but, after the first trial, the two humuses were diluted at the rate of 1 to 10,000,000. The latter dilution gave a much better distribution of colonies on the plates.

In the May 21 trial, the plates made from humus were so very alkaline that when the covers were raised, the plates gave off a strong ammonia odor. Red litmus paper held inside the plates for a few minutes turned blue. No mold colonies showed on these plates. While the actinomyces colonies were not as numerous as the colonies of other organisms, it was suspected that they contributed much of the ammonia. When transfers were made to tubes filled with nutrient agar plus sufficient brom-thymol blue to give them color, the alkalinity after a few days reached pH 7.5 and red litmus paper gave the blue reaction. Other bacterial ammonifiers probably take part also in this protein destruction of the humus but the *Actinomyces albus* group is one of the most important agents.

A number of trials were made during the following summer (1944) with results, in general, very similar to those obtained in 1943. New humus beds of buckwheat, potato, and winter rye had been made, the first two in the fall of 1943 and the winter rye on May 30, 1944. The summer of 1944 was very dry and hot and the rainfall was frequently not sufficient to hasten the destruction of organic remains. The summer of 1943, on the other hand, had been very wet so that the soil and humus received enough moisture to keep the organic changes going at full speed.

One example (taken on July 24, when potato tubers were forming) will be sufficient for this year (Table 5).

Table 5. Alkalinity of, and Numbers and Types of Organisms Present in, Soil and Buckwheat and Potato Humus, July 24, 1944

Material	pH	Organisms present			
		Bacteria	Actinomycetes	Molds	Yeasts
Soil (1-inch depth)	7.0	17,500,000	1,100,000	1,900,000	18,000
Soil (2- to 3-inch depth)	6.9	18,000,000	700,000	*	*
Buckwheat humus (made 1943)	7.1	1,950,000,000	23,000,000†	4,000,000	1,300,000
Potato humus (made 1942)	7.0	3,070,000,000	62,000,000†	5,000,000	1,700,000

* Not counted.
† All *Act. albus.*

The field had been limed since the 1943 trials, which explains the increased alkalinity of the soil.

The potato humus from the bed made in the fall of 1942 was quite well disintegrated by 1944, because of the action of various

microorganisms and the activities of earthworms. The humus layer was full of earthworm holes and castings, having been worked over quite well, but the actinomycetes continued to be very numerous, passage through the earthworms having had no effect on them.

The following facts seem to be indicated by the results obtained from the humus beds:

1. The predominant actinomyces in the one-year-old humus was *Act. albus,* while in soil which contained only very old humus, *Act. chromogenus* was more abundant.

2. Humus was always much more alkaline than the surrounding soil.

3. Actinomycetes cultures produced a pH of 7.5 and had a strong smell of ammonia, from which it may be deduced that these organisms were one of the chief sources of the alkalinity of the humus. Later, molds were very abundant in the humus and they also contributed to the alkalinity.

4. The humus was always very wet as compared to the soil and this moisture helped the continued growth of the actinomycetes and molds.

THE INCIDENCE OF SCAB ON POTATOES GROWN ON HUMUS-INOCULATED SOIL

Potatoes were grown in 1941 on a plot which had yielded 100 percent scabby tubers. After the harvest and entire removal of the crop of scabby tubers, the tops and attached roots were gathered into a large compost heap on this infected land. By the following May, the softer parts, such as the leaves, had rotted, but large fragments of the partially destroyed stems and roots could still be picked from the compost.

This compost was used as an inoculum in 1942, a shovelful being placed in each hill with the seed piece. Six short rows were set out, each containing 25 plants. Three of the rows were inoculated, while the three alternating rows were left uninoculated as checks.

These rows were on land only a few feet from the old plot and on the same type of soil. However, no potatoes had been grown on this soil for over 35 years, and it was believed to be quite scab-free. The seed had been disinfected and only a liberal application of commercial fertilizer was applied. At the same time, potatoes were still being grown on the old plot from which the tops and tubers had been harvested and which was known to produce 100 percent scabby tubers.

In making the inoculation, some of the soil that had produced the very scabby tubers was necessarily carried over with the humus into the hill. Since, however, no crop except potatoes had been grown

on this infected soil for at least six years, all plant remains in it may be expected to be those of the potato. The tubers had been removed each year so that any broken-up bits of plant débris in the soil would be older than those in the humus pile made the preceding season, but otherwise similar. Even if the humus pile had been built on clean soil, some soil from the older infected plot would necessarily have been carried over into the pile on the roots and underground stems, the only difference being that a smaller amount of the old infected soil would be involved. A potato humus pile simply cannot be made that will not contain some old soil.

Tubers from inoculated and uninoculated hills were brought into the laboratory and a number of them photographed. Some of these tubers are shown in Figure 12. The primed figures (A′ and B′) indicate that the tubers were from inoculated plants. Lots A and A′ were each from the fifth hill in their row; B and B′ were each from the twenty-fourth hill.

The potatoes shown in Figure 12 are typical of the results obtained. Inoculation resulted in a heavy scab infection in every hill, infection which was even worse than that obtained in the old infested area. Occasional scabs appeared on tubers from the control rows, but such an infection might be expected since the same tools were used in cultivating all six rows and under field conditions it is not possible to practice bacteriological technic. No photographs were taken of the tubers from hills in the old plot but in no cases were the infections as large or as numerous as on the inoculated hills.

The reasons for this increase in scab are to be found not only in the scab organisms which the potato humus introduced in the vicinity of the growing tubers but also in the physical and chemical properties of that humus.

As has already been shown in one of the preceding sections, potato humus is destroyed very slowly in the soil and potato remains of the preceding year are largely undestroyed by biological activities during all of the following growing season. On the other hand, buckwheat or winter rye humus is largely decomposed before the young tubers can be infected. The difference seems to lie in the more fragile construction of the buckwheat and winter rye plants.

Even in the driest part of the summer, the potato humus is quite moist. On July 16, 1943, the potato humus had a moisture content of 70 percent, while the adjacent soil had only 7 percent.

This undecayed potato humus contains millions of *Actinomyces albus* in every gram and these organisms are at all times very marked ammonia producers. In the analysis carried out in 1943, the potato humus remained always at least neutral and at times was as alkaline as 7.5, while the soil had a pH of only 6.4.

Growers have long recognized that potatoes grow best and are also scabbiest on a soil that has a pH between 5.4 and 6.6. In this connection the work of two surveys might be quoted. Blodgett and Cowan (1935) stated that "Scab increased with increased alkalinity

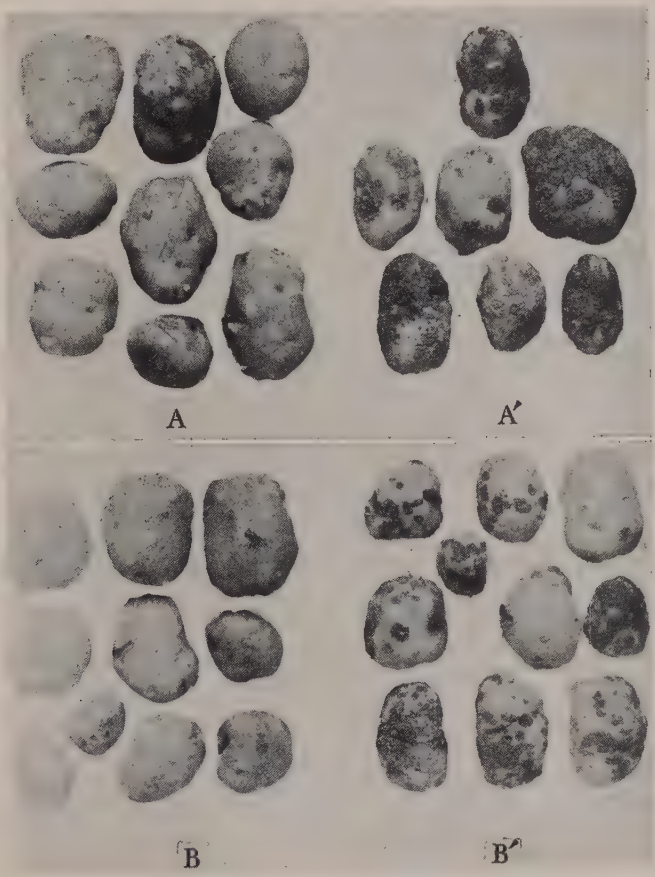

FIG. 12. Potatoes from four hills. Lots A and B are from hills 5 and 24 in an uninoculated row. A′ and B′ are from hills 5 and 24 in an inoculated row, in which a spadeful of potato humus was placed in each hill with the seed piece.

of the soil to approximately pH 6.6 but as the alkalinity was increased beyond this point there was a tendency for the scab to decrease." Blodgett and Howe (1934) said that in New York State the soils with a reaction of pH 5.45-7.4 had the most scab. Cook and Nugent (1942), using acid soils, found the amount of scab to be correlated

with the soil reaction and that very significant increases in scabbing occurred if the fertilizers used were non-acid forming.

The scab-producing actinomycetes grow best in a slightly acid medium since they themselves are strong ammonia producers and alkalinity seems to be an important limiting factor in their growth. Fresh manure has always been considered to be especially bad as a fertilizer for potatoes, supposedly because of its continued alkalinity and the humus which it produces. Lime has been put in the same category as manure, the calcium ion in this chemical being held responsible for the increased actinomyces pathogenicity. Lime, as is well known, accelerates the destruction of humus in the soil. The suggestion may be made that the vigor of the organisms which destroy the pectin layer which holds the cells together is stimulated by the lime. Thirty percent of the potato plant ash is calcium oxide.

The production of ammonia by actinomycetes and the resultant alkalinity are only incidental and not essential to pathogenicity. In fact, pronounced alkalinity may act as a retarder on scab-producing activities, as the scab investigators just quoted have found.

Infection of young potato tubers is dependent on the ability of the actinomycetes to dissolve the pectin between the cell walls of these growing organs. Fresh manure, lime, and potato humus would serve the purpose of increasing the vigor of the organisms.

A new and quite different viewpoint has been suggested by Schroeder and Albrecht (1942), whose experiments indicated a relationship between the potassium-calcium ratio and the amount and severity of scabbing. They found that when potassium was used to excess in the fertilizer and no calcium, or too little calcium, was added, the percentage of scabby tubers increased.

The water-holding characteristics of potato humus and its persistence in the soil serve a quite different purpose. The scab-producing actinomycetes may live for years in humus. The water contained in the humus enables them not only to survive but to continue active growth so that they are ready to push into a growing tuber without the delay for germination which would otherwise occur.

PRACTICAL APPLICATIONS OF THIS WORK

The infection of parts of the potato plant other than tubers has not previously been emphasized. In this paper, such infection has been demonstrated by inoculations and an examination of the stems and roots.

The actinomycetes in the soil have been shown to come from mycelium or resting spores in the decaying plant remains. If potato humus is left on the field it will serve as a carrier of scab-producing

actinomycetes. All potato plants should therefore be removed if the land is known to be favorable to scab, even though their removal means a serious loss in humus. The potato tops should be removed (as far as is practicable) and used on another field where other crops will be grown. However, many of the underground stolons and roots cannot be gathered by any cheap method and so they must be left.

The view that potato scab is spread by infected tubers has cost potato growers millions of dollars in disinfecting seed potatoes. The writer, thirty years ago, pointed out the futility of such disinfections (Lutman and Cunningham, 1914): "If scabbing of the tubers is only occasional and the spots are small but little gains are to be derived from seed disinfection. . . . Not only is the benefit slight, but there is always the chance of injuring the seed to some extent even though the operation is carefully done." But in spite of the evidence at that time produced, this expensive and useless procedure continued to be recommended. Now, after 30 years, the War Emergency Committee of American plant pathologists has abandoned this recommendation of seed tuber disinfection (Clayton, 1944).

SUMMARY

1. When potato stems from scab-infected plots were examined, the vertical portion sometimes showed typical browned and cracked scab lesions, and many of the numerous lenticels were browned and slightly enlarged. Brown, elongated lenticels were frequent on the white stolons which terminated in badly scabbed tubers. Scabs found on stems and stolons were the same as those which develop on tubers.

2. Young potato roots are normally white but, as they become longer and larger, they show brown spots of varying sizes. Such spots were found located in the cortical regions of the roots. Tangential and cross sections of the roots showed some cell walls that were browned and swollen while others were thin and transparent. The former type seemed to enclose filaments which excreted pigment into the adjacent cytoplasm. These filaments are the same as the intercellular filaments that are visible in the skin of young potato tubers near young scab lesions and are without doubt the growth of chromogenic actinomycetes. The walls of all cortical cells had a very complicated structure, but only a small number were brown.

3. Soil actinomycetes were grown in a differential liquid medium composed of pectin, arabinose (pectin sugar), and inorganic salts in small smears on cover glasses. This medium was also used as a gel by the addition of 0.5 percent agar. This medium has a pH of 5.6 and the pectin is, therefore, unavailable except to a very few bacteria. The growth of the very numerous ammonifying soil bacteria was suppressed but many molds and yeasts grew on it.

The actinomyces germ tubes arose in all cases from the interior of very thoroughly disintegrated potato humus particles; no conidia (nor germ tubes from conidia) were observed. These bits of humus were either colorless or stained a dark brown, but in all cases they were amorphic and very granular. In a few small transparent bits, germination seemed to have occurred at both ends of a short piece of mycelium. This humus which was made from previous potato crops was from two to seven years old.

4. Similar germinations were obtained from finely pulverized potato humus (mostly leaves) made the preceding year. In these cases the fragments could still be recognized as plant cells and walls but the exact origins of the germinating hyphae growing from them could not be determined. The diameters of the actinomyces hyphae growing from plant organs varied, suggesting that more than one species were to be found inside the potato plant.

5. Few actinomyces germinations could be obtained from the soil before the first week in June, when the soil had a temperature of about 16 to 18 degrees C. The number of germinations increased until about the middle of July. They were checked and almost suspended by a period of drought when the moisture content of the soil fell to about 6 percent of its dry weight. Heavy rains after this period did not restore the rapidity or number of germinations for nearly two months. Germinations continued until December when the last trials were made. This germination pattern follows the scab infection temperatures that have been worked out under control conditions. The drought was a disturbing factor. A low moisture content in the soil delayed germinations from 12 to 72 hours.

6. The persistence of actinomycetes in soil, especially in soil containing a high percentage of humus, was shown by the isolation of millions of *Act. chromogenus* per gram from soil samples which had been kept in glass-stoppered bottles for seven years. They were still numerous in every bottle in which the soil was moist.

7. Potato humus, even if it contained no pathogenic organisms, would serve to aggravate scab since it was found to contain 46 percent moisture at a time when the soil had no more than 23 percent, and it is quite alkaline in reaction. This alkalinity is ascribed largely to the very numerous *Actinomyces albus* it contains. Moreover, potato humus disintegrates very slowly in the soil and fragments of stems and masses of leaves could still be found the following growing season. On the other hand, in buckwheat humus, all leaves rotted between October and July and by the first of the following September only the bases of the stems could be identified. Winter rye humus decayed even faster. Both buckwheat and winter rye humus were

alkaline during the early phases of their dissolution but the thin leaves and hollow stems of the buckwheat went to pieces very rapidly.

8. One experiment was conducted in which potatoes were grown in soil which had been inoculated with infected potato humus. The soil was quite free from scab-producing actinomycetes. It had a pH of 6.4. The humus was made from potato remains of the preceding years, from plants which had grown on soil that was badly infected by the pathogenic species. The tubers produced in soil receiving these humus additions were much scabbier than those grown in adjacent uninoculated soil.

9. The scab actinomycetes live in the soil in humus derived from parts of the potato plant other than the tubers. To control scab, therefore, all potato remains should be removed from land known to be favorable to scab as far as is practicable after harvest.

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